COMMUNICATION

Frozen Acrylamide Gels as Dynamic Nuclear Polarization Matrices.

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Abstract: We show that aqueous acrylamide gels can be used to provide dynamic nuclear polarization (DNP) NMR signal enhancements of around 200 at 9.4 T and 100 K. The enhancements are shown to increase with cross linker concentration and low concentrations of the AMUPol biradical. We show that this DNP matrix can be used in situations where conventional incipient wetness methods fail, such as to obtain DNP surface enhanced NMR spectra from inorganic nanoparticles. In particular, we obtain ^{113}Cd spectra from CdTe-COOH NPs in minutes. The spectra clearly indicate a highly-disordered cadmium rich surface.

Dynamic nuclear polarization (DNP) is a rapidly expanding method that can provide an increase in solid-state NMR signal intensity by 2 orders of magnitude,[1] thereby enabling atomic-level characterization of systems that were previously completely inaccessible.[1a, 1c, 2] DNP works by transferring polarization from unpaired electrons to nearby nuclei. This is enabled in diamagnetic samples by doping with a stable radical as a polarization source. Additionally, a medium is required to transfer polarization by spin diffusion from the source to the nuclei of interest and to distribute homogeneously the polarizing agents. This typically leads to formulations of frozen solutions of organic nitroxide based biradicals (TEKPol,[3] AMUPol,[4] TOTAPOL[5]…) in glass forming mixtures which can either be aqueous water/glycerol or water/DMSO, or a range of organic solvents from 1,1,2,2-tetrachloroethane[6] to *ortho*-terphenyl.[7] Substrates are either directly dissolved in the solution,[8] or for materials samples impregnated with the polarizing solution.[9] Formation of a glass has proven to be an essential requirement to avoid separation or precipitation effects upon freezing leading to poor DNP performance.[3, 10]

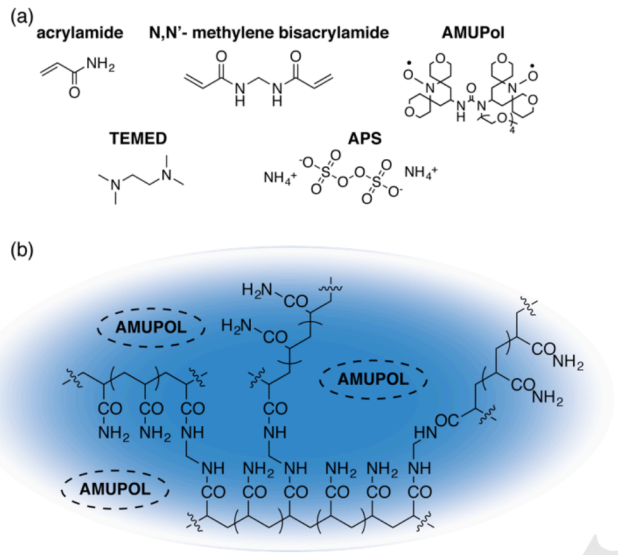
However, there is still today essentially only one water based formulation. The most popular DNP matrix is glycerol-d_8/D_2O/H_2O in a ratio of (6/3/1 v/v) which has been empirically optimized to give the best enhancements (typically around 200 at 9.4 T and 100 K), and which is often referred to as "DNP Juice." Even the organic solvents, which have a broad range of properties and are compatible with many substrates, encounter problems in systems prone to aggregation, with nanoparticles being a prime example that have not so far been amenable to study in any ordinary solvents.[11]

The importance of these limitations is demonstrated if we consider the work that has been done to find alternatives. De Paëpe and others have used so-called matrix-free DNP methods to characterize liposomes[12] and small proteins[13] or membrane proteins.[14] As well as in the case of in-vivo magnetic resonance imaging of silicon nanoparticles their surface composed of defect-bound electrons do not require additional matrix.[15] To prevent aggregation of colloidal solutions of nanoparticles (NPs) at cryogenic temperatures, they have been dispersed in mesoporous silicas,[11], whereas incipient wetness impregnation of NPs might work in some case such as CeO_2.[16] Pure water has been polarized in hybrid solids.[17] In the case of reactive surface organometallic complexes, methods were developed to avoid contact between free radical and reactive site (using materials with small pores or bigger radicals) so as to prevent reaction.[18] Others have separated the radical from the substrate by creating dendrimers around the polarizing source.[18b] Micelle or supramolecular based systems have also been considered.[19] These methods are often not trivial to implement, and they all lead to significant reductions in DNP efficiency as compared to ordinary DNP Juice.

Here, we introduce a new water-based matrix for DNP using acrylamide based gels. Polyacrylamide gels, which are easy to make and widely used, for example in electrophoresis,[20] are made of a cross-linked network formed of polymer chains which provides interstitial spaces filled with water.[21] Notably they can undergo large deformations such as significant reversible swelling or collapsing to accommodate substrates.[21a, 22] We show that polyacrylamide gels can be used to achieve enhancements of over 200 at 9.4 T and 100 K. We also demonstrate that hydrophilic carboxylic acid capped CdTe (CdTe-COOH) quantum dots can be characterized with DNP using this matrix.

Frozen acrylamide gels for DNP enhanced NMR spectroscopy. Scheme 1 shows the chemical structures of the compounds used to form the polyacrylamide gel. The monomer, acrylamide (acryl), is copolymerized in D_2O with the crosslinker, *N*, *N'*- methylene bisacrylamide (bisacryl), using ammonium persulfate (APS) as the initiator and tetramethylethyldiamine (TEMED) as a redox activator. We investigated three different acryl:bisacryl ratios: 37.5:1 (2.7 % crosslinker), 29:1 (3.3 % crosslinker) and 19:1 (5 % crosslinker) which we refer to as gels **A**, **B** and **C** respectively. Varying the monomer to cross linker ratio has the known effect of varying the interstitial space in the gel,

which can be thought of in terms of a pore size.[23] In order to remove excess initiators (that can react with the polarizing agents) and residual monomers, the gel is then purified using the breathing technique of Willner and coworkers (see SI),[24] initially developed to uniformly distribute gold NPs in the gel matrix.[22d, 24-25]



Scheme 1. (a) Polyacrylamide components: monomer acrylamide; cross-linker N,N'- methylene bisacrylamide; initiator ammonium persulfate and accelerator tetramethylethyldiamine. (b) DNP Jelly.

Here we first add acetone to the gel, which collapses and turns as a white soft solid (see Figure S2 (b)) as the water containing APS and TEMED is expulsed. The soft solidl is then immersed in an excess of D_2O, in which it swells back to its initial state, regaining a translucent gel aspect (see Figure S2 (c)). This breathing cycle is repeated four times. Once the gel is pure, it is immersed in a 1:1 w/v solution of varying concentrations of AMUPol in D_2O for one hour (Scheme 1 (b)), allowing diffusion of the free radical into the gel, leading to a polarizing gel that can be used for DNP experiments, and that we refer to as DNP Jelly. (Refer to SI for a more complete description of the method.)

Correlation between the cross-linker and radical concentrations, and ε.

Figure 1(a) shows the ε_H, $\varepsilon_{C=O,\,CP}$, and $\varepsilon_{alkyl,\,CP}$ factors obtained for gels **A**, **B**, and **C**. They were obtained by comparing integration of the resonances of interest for the gel spectra acquired with and without microwave irradiation and correspond to the carbonyl and alkyl functionalities of the propionamide units. The ^{13}C CPMAS NMR spectra of the gels are shown in Figure S3. ε increases as the acryl:bisacryl ratio decreases for gels **A**, **B** and **C** respectively: from 119(35) to 192(59) (for the carbonyls), and 75(11), to 131(26) (for the alkyls) and from 54 to 75 for ^1H. The ratio between monomer and cross-linker is known to have an influence on the pore size in the gel, larger pore sizes are found for low crosslinker concentrations. Here the pore size decreases from gel **A**>**B**>**C,** and varies between 41 to 10 nm, respectively, as estimated using the approach of Holmes et al.).[23] Thus we can hypothesise that smaller pores improve either the homogeneous dispersion of the free radical in the gel, or the quality of the glass formed upon cooling, and leads to better DNP enhancements.[26]

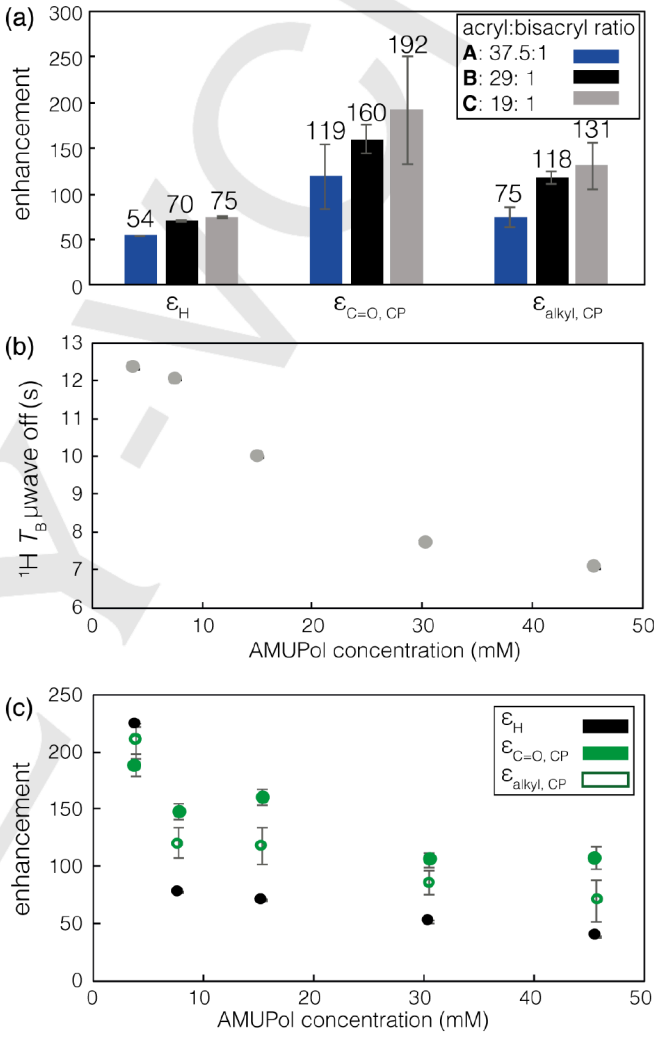


Figure 1. (a) DNP $\varepsilon_{C=O,\,CP}$, $\varepsilon_{alkyl,\,CP}$ and ε_H factors of the gels as a function of acryl: bisacryl ratio for polyacrylamide gel in 10 mM AMUPol/D_2O. (b) T_B acquired with μwave off as a function of the AMUPol concentration in *DNP jelly* **C**. (c) Plot of the ^{13}C and ^1H DNP enhancement as a function of the effective AMUPol concentration in gel C (acryl:bisacryl 19:1 ratio).

In Figure 1 (a), we note a significant difference between the proton enhancements measured directly (ε_H) or measured through cross polarization ($\varepsilon_{C,\,CP}$) for all three gels. We believe that the higher factor observed with CP indicates that the AMUPol has an affinity for the polymer, and thus higher hyperpolarization is obtained for protons which are inside the gel network. The lower ε for the total ^1H content reflects an average between ^1H enhancements of the gel network and those of excess water outside the network where

the local AMUPol concentration would then be lower and DNP less efficient. We observe good DNP ε for frozen DNP Jelly. The white opaque color might correspond to micro-domains of water crystalizing (see SI, Figure S5). The DNP process is not affected since they are micro-domains, i.e. radical concentrated zones are minimal.

Figure 1b and c show ^{1}H build-up times (T_B) and ε_H, $\varepsilon_{C=O,\,CP}$, $\varepsilon_{alkyl,\,CP}$ for gel **C** as a function of the free radical concentration. $T_{B,\,off}$ decreases with increasing radical concentration in line with expectations.[4, 27] The maximum DNP enhancement is obtained at lower radical concentrations than for conventional glycerol-d_8/D_2O/H_2O formulations. The concentration of AMUPol in the gel sample (gel type C) after diffusion has been calculated by measuring the concentration of remaining AMUPol in the supernatant with quantitative liquid-state NMR (see SI for the complete procedure). The concentration curve is broader than those previously reported for other nitroxide biradicals.[6, 28]

The best formulation found here for gel **C** with 3.8 mM AMUPol in D_2O yields ε_C of around 200, which compares favourably to the gold standard 10 mM AMUPol glycerol-d_8/D_2O/H_2O (6/3/1 v/v) that provides a typical ε of 250 under the same conditions (see Figure S4) at 9.4 T. Note that if we assume no proton/deuterium exchange for the alkyl chains, then the ^{1}H concentration in DNP Jelly is the same (15 M) as glycerol-d_8/D_2O/H_2O (6/3/1 v/v).[29]

^{113}Cd DNP of CdTe-COOH *NP*. As discussed above, and reported in the literature,[11] spectra of the surface of quantum dots (QDs) are particularly challenging to acquire using conventional NMR due to low concentrations. The application of DNP surface Enhanced NMR Spectroscopy (SENS)[9] is hindered by aggregation of NPs upon freezing of colloidal solutions at 100 K.[11] The capacity of polyacrylamide gels as soft matter to adapt to a range of impregnated substrates by breathing properties makes them potentially very well suited for DNP SENS of NPs. Here hydrophilic CdTe-COOH core-type QDs (λ_{em}= 520 nm) were therefore dispersed into a formulation of DNP Jelly with the breathing technique[22d] (see SI for method). ^{1}H-^{113}Cd CP DNP SENS experiments where then performed to obtain surface selective spectra, while direct excitation experiments permit the entire NP to be analysed.

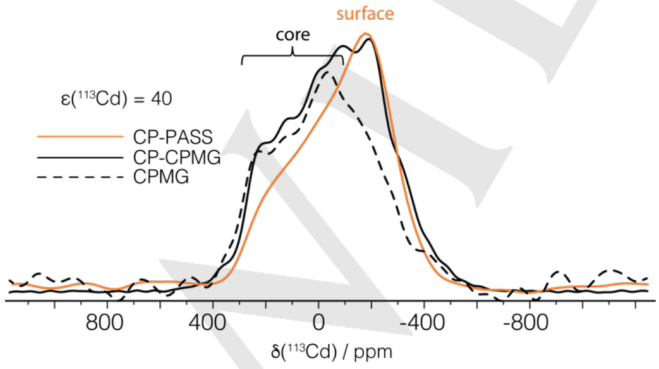


Figure 2. ^{113}Cd DNP CP-CPMG (black) and CPMG (dashed), as well as isotropic projection of 2D CP-PASS (orange) spectrum of CdTe-COOH NP dispersed in gel **C**. Spinning sidebands can be depicted (MAS of 10 kHz) in the (CP-)CPMG experiments.

As shown in Figure 2, a ^{113}Cd CP-CPMG spectrum can be acquired in minutes with DNP, with an ε_{Cd} of 40 (see SI for the μwave off spectrum). The use of a CPMG experiment provides a further ε of 8 as compared to simple CP-echo experiment. The distribution of ^{113}Cd chemical shifts observed in Figure 2 in this way (δ) is very broad (δ(^{113}Cd) from 400 to -400 ppm) which signifies Cd atoms at the surface in a broad range of different disordered coordination environments.[30] Such broad Cd spectra have previously been observed for Cd-rich surfaces in CdS NPs (0 to -750 ppm).[30b] A two-dimensional sideband (PASS) experiment[31] of the NPs dispersed in the gel was acquired to obtain the isotropic spectrum devoid of spinning sidebands (Figure 2, orange trace), and is slightly narrower than the CP-CPMG spectrum, with the distribution of δ(^{113}Cd) concentrated towards low frequency range, as expected for surface atoms. When the direct excitation experiment (with CPMG) is compared to the surface CP spectrum, one can see a slight shift to higher frequencies, as expected since relatively more signal from the core atoms is enhanced, however the width of the spectrum remains broad. This suggests a Cd rich surface and Te rich core for the particles.

In conclusion, we have prepared a series of three polyacrylamide gels with different acryl: bisacryl ratios and studied their performance as a matrix for DNP experiments at 9.4 T, 100 K using the AMUPol biradical. We observed that high DNP ε (200) are obtained when higher cross linker concentration (acryl: bisacryl 19:1 ratio) is used to form the polymer matrix along with low concentration of radical (3.8 mM). A lower concentration of AMUPol (3.8 mM) is necessary in DNP Jelly to obtain a competitive ε compared to 10 mM of AMUPol in glycerol-d_8/D_2O/H_2O (6/3/1 v/v). Finally, we have demonstrated these gels as a soft matter can be used as a medium to study material systems which could not be studied before using conventional NMR or conventional DNP formulations. We determine the structure of CdTe-COOH QDs to feature a disordered Cadmium rich surface with Cd atoms in a range of coordination environments using DNP Jelly, where the ^{113}Cd spectra were acquired in minutes.

Experimental Section

NMR Experiments: All DNP NMR experiments were acquired on a 263 GHz/400 MHz Bruker Avance I or III spectrometer equipped with a low temperature magic angle spinning probe operating at ^{1}H, ^{13}C, ^{113}Cd Larmor frequencies of 400 MHz, 125 MHz, and 88 MHz, respectively. Sample were packed in 3.2 mm o.d. sapphire rotors for experiments performed at low temperature (90-100K). Further details for acquisition parameters can be found in SI for CPMAS, (CP-)CPMG and PASS. *Gel synthesis:* Polyacrylamide reagents were from BioRad or Sigma Aldrich and used without further purification. Further details on gel preparation and cleaning can be found in SI. *CdTe samples:* CdTe core-type COOH capped QDs were bought from Aldrich and used without

further purification. Details on the sample formulation with the gel can be found in SI.

Acknowledgements

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Keywords: Dynamic nuclear polarization, polyacrylamide gel, nanoparticles, quantum dots.

[1] aA. J. Rossini, A. Zagdoun, M. Lelli, A. Lesage, C. Copéret, L. Emsley, *Acc. Chem. Res.* **2013**, *46*, 1942-1951; bT. Kobayashi, F. A. Perras, I. I. Slowing, A. D. Sadow, M. Pruski, *ACS Catal.* **2015**, *5*, 7055-7062; cY. Su, L. Andreas, R. G. Griffin, *Annu. Rev. Biochem.* **2015**, *84*, 465-497; dA. N. Smith, M. A. Caporini, G. E. Fanucci, J. R. Long, *Angew. Chem. Int. Ed. Engl.* **2015**, *54*, 1542-1546; eQ. Z. Ni, E. Daviso, T. V. Can, T. M. Markhasin, R. J. Temkin, J. Herzfeld, R. G. Griffin, *Accounts of Chemical Research* **2013**, *46*, 1933-1941.

[2] aR. G. Griffin, T. F. Prisner, *Phys. Chem. Chem. Phys.* **2010**, *12*, 5737-5740; bP. Berruyer, M. Lelli, M. P. Conley, D. L. Silverio, C. M. Widdifield, G. Siddiqi, D. Gajan, A. Lesage, C. Coperet, L. Emsley, *J. Am. Chem. Soc.* **2017**; cD. Lee, H. Takahashi, A. S. Thankamony, J. P. Dacquin, M. Bardet, O. Lafon, G. D. Paepe, *J. Am. Chem. Soc.* **2012**, *134*, 18491-18494; dA. R. Mouat, C. George, T. Kobayashi, M. Pruski, R. P. van Duyne, T. J. Marks, P. C. Stair, *Angew. Chem. Int. Ed. Engl.* **2015**, *54*, 13346-13351; eR. P. Sangodkar, B. J. Smith, D. Gajan, A. J. Rossini, L. R. Roberts, G. P. Funkhouser, A. Lesage, L. Emsley, B. F. Chmelka, *J. Am. Chem. Soc.* **2015**, *137*, 8096-8112; fK. K. Frederick, V. K. Michaelis, B. Corzilius, T. C. Ong, A. C. Jacavone, R. G. Griffin, S. Lindquist, *Cell* **2015**, *163*, 620-628; gJ. Becker-Baldus, C. Bamann, K. Saxena, H. Gustmann, L. J. Brown, R. C. Brown, C. Reiter, E. Bamberg, J. Wachtveitl, H. Schwalbe, C. Glaubitz, *Proc Natl Acad Sci U S A* **2015**, *112*, 9896-9901.

[3] A. Zagdoun, G. Casano, O. Ouari, M. Schwarzwalder, A. J. Rossini, F. Aussenac, M. Yulikov, G. Jeschke, C. Coperet, A. Lesage, P. Tordo, L. Emsley, *J. Am. Chem. Soc.* **2013**, *135*, 12790-12797.

[4] C. Sauvee, M. Rosay, G. Casano, F. Aussenac, R. T. Weber, O. Ouari, P. Tordo, *Angew. Chem. Int. Ed. Engl.* **2013**, *52*, 10858-10861.

[5] C. Song, K.-N. Hu, C.-G. Joo, T. M. Swager, R. G. Griffin, *J. Am. Chem. Soc.* **2006**, *128*, 11385-11390.

[6] A. Zagdoun, A. J. Rossini, D. Gajan, A. Bourdolle, O. Ouari, M. Rosay, W. E. Maas, P. Tordo, M. Lelli, L. Emsley, A. Lesage, C. Coperet, *Chem. Commun.* **2012**, *48*, 654-656.

[7] T.-C. Ong, M. L. Mak-Jurkauskas, J. J. Walish, V. K. Michaelis, B. Corzilius, A. A. Smith, A. M. Clausen, J. C. Cheetham, T. M. Swager, R. G. Griffin, *The Journal of Physical Chemistry B* **2013**, *117*, 3040-3046.

[8] aC. Song, K.-N. Hu, C.-G. Joo, T. M. Swager, R. G. Griffin, *J. Am. Chem. Soc.* **2006**, *128*, 11385-11390,; bV. S. Bajaj, C. T. Farrar, M. K. Hornstein, I. Mastocsky, J. Vieregg, J. Bryant, B. Eléna, K. E. Kreischer, R. J. Temkin, R. G. Griffin, *J. Magn. Reson.* **2003**, *160*, 85-90.

[9] A. Lesage, M. Lelli, D. Gajan, M. A. Caporini, V. Vitzthum, P. Miéville, J. Alauzun, A. Roussey, C. Thieuleux, A. Mehdu, G. Bodenhausen, C. Copéret, L. Emsley, *J. Am. Chem. Soc.* **2010**, *132*, 15459-15461.

[10] A. Barnes, G. De Paëpe, P. van der Wel, K. N. Hu, C.-G. Joo, V. S. Bajaj, M. L. Mak-Jurkauskas, J. R. Sirigiri, J. Herzfeld, R. J. Temkin, R. G. Griffin, *Appl. Magn. Reson.* **2008**, *34*, 237-263.

[11] L. Piveteau, T.-C. Ong, A. J. Rossini, L. Emsley, C. Copéret, M. V. Kovalenko, *J. Am. Chem. Soc.* **2015**, *137*, 13964-13971.

[12] C. Fernandez-de-Alba, H. Takahashi, A. Richard, Y. Chenavier, L. Dubois, V. Maurel, D. Lee, S. Hediger, G. De Paepe, *Chemistry* **2015**, *21*, 4512-4517.

[13] H. Takahashi, S. Hediger, G. De Paepe, *Chem Commun (Camb)* **2013**, *49*, 9479-9481.

[14] E. S. Salnikov, H. Sarrouj, C. Reiter, C. Aisenbrey, A. Purea, F. Aussenac, O. Ouari, P. Tordo, I. Fedotenko, F. Engelke, B. Bechinger, *J. Phys. Chem. B* **2015**, *119*, 14574-14583.

[15] aJ. W. Aptekar, M. C. Cassidy, A. C. Johnson, R. A. Barton, M. Lee, A. C. Ogier, C. Vo, M. N. Anahtar, Y. Ren, S. N. Bhatia, C. Ramanathan, D. G. Cory, A. L. Hill, R. W. Mair, M. S. Rosen, R. L. Walsworth, C. M. Marcus, *ACS Nano* **2009**, *3*, 4003-4008; bM. C. Cassidy, H. R. Chan, B. D. Ross, P. K. Bhattacharya, C. M. Marcus, *Nat. Nanotechnol.* **2013**, *8*, 363-368.

[16] M. A. Hope, D. M. Halat, P. C. Magusin, S. Paul, L. Peng, C. P. Grey, *Chem. Commun.* **2017**, *53*, 2142-2145.

[17] aD. Gajan, M. Schwarzwalder, M. P. Conley, W. R. Gruning, A. J. Rossini, A. Zagdoun, M. Lelli, M. Yulikov, G. Jeschke, C. Sauvee, O. Ouari, P. Tordo, L. Veyre, A. Lesage, C. Thieuleux, L. Emsley, C. Coperet, *J. Am. Chem. Soc.* **2013**, *135*, 15459-15466; bD. Gajan, A. Bornet, B. Vuichoud, J. Milani, R. Melzi, H. A. van Kalkeren, L. Veyre, C. Thieuleux, M. P. Conley, W. R. Gruning, M. Schwarzwalder, A. Lesage, C. Coperet, G. Bodenhausen, L. Emsley, S. Jannin, *Proc Natl Acad Sci U S A* **2014**, *111*, 14693-14697; cA. J. Rossini, A. Zagdoun, M. Lelli, D. Gajan, F. Rascon, M. Rosay, W. E. Maas, C. Coperet, A. Lesage, L. Emsley, *Chem. Sci.* **2012**, *3*, 108-115.

[18] aE. Pump, J. Viger-Gravel, E. Abou-Hamad, M. K. Samantaray, B. Hamzaoui, A. Gurinov, D. H. Anjum, D. Gajan, A. Lesage, A. Bendjeriou-Sedjerari, L. Emsley, J.-M. Basset, *Chem. Sci.* **2016**; bW.-C. Liao, T.-C. Ong, D. Gajan, F. Bernada, C. Sauvee, M. Yulikov, M. Pucino, R. Schowner, M. Schwarzwalder, M. R. Buchmeiser, G. Jeschke, P. Tordo, O. Ouari, A. Lesage, L. Emsley, C. Coperet, *Chem. Sci.* **2016**.

[19] aM. Lelli, A. J. Rossini, G. Casano, O. Ouari, P. Tordo, A. Lesage, L. Emsley, *Chem Commun (Camb)* **2014**, *50*, 10198-10201; bK. G. Valentine, G. Mathies, S. Bedard, N. V. Nucci, I. Dodevski, M. A. Stetz, T. V. Can, R. G. Griffin, A. J. Wand, *J. Am. Chem. Soc.* **2014**, *136*, 2800-2807; cJ. Mao, D. Akhmetzyanov, O. Ouari, V. Denysenkov, B. Corzilius, J. Plackmeyer, P. Tordo, T. F. Prisner, C. Glaubitz, *J. Am. Chem. Soc.* **2013**, *135*, 19275-19281.

[20] B. Hames, Oxford University Press, Oxford **1998**.

[21] aI. Wichterle, D. Lim, *Nature* **1960**, *4706*, 117-118; bM. Nic, J. Jirat, B. Kosata, *IUPAC* **2014**, doi:10.1351/goldbook.G02600.

[22] aD. Nicoli, C. Young, T. Tanaka, A. Pollak, G. Whitesides, *Macromolecules* **1983**, *16*, 887-890; bY. Osada, J.-P. Gong, *Adv. Mater.* **1998**, *10*, 827-837; cM. Shibayama, Y. Shirotani, H. Hirose, S. Nomura, *Macromolecules* **1997**, *30*, 7307-7312; dP. Thoniyot, M. J. Tan, A. A. Karim, D. J. Young, X. J. Loh, *Adv. Sci.* **2015**, *2*, 1400010.

[23] D. L. Holmes, N. C. Stellwagen, *Electrophoresis* **1991**, *12*, 612-619.

[24] V. Pardo-Yissar, R. Gabai, A. N. Shipway, T. Bourenko, I. Willner, *Adv. Mater.* **2001**, *13*, 1320-1323.

[25] aJ. H. Holtz, S. A. Asher, *Nature* **1997**, *389*, 829-832; bC. Wang, N. T. Flynn, R. Langer, *Adv. Mater.* **2004**, *16*, 1074-1079.

[26] D. Baudouin, H. A. van Kalkeren, A. Bornet, B. Vuichoud, L. Veyre, M. Cavailles, M. Schwarzwalder, W. C. Liao, D. Gajan, G. Bodenhausen, L. Emsley, A. Lesage, S. Jannin, C. Coperet, C. Thieuleux, *Chem. Sci.* **2016**.

[27] M. Rosay, Massachusetts Institute of Technology (Massachusetts), **2001**.

[28] aH. Takahashi, C. Fernandez-de-Alba, D. Lee, V. Maurel, S. Gambarelli, M. Bardet, S. Hediger, A. L. Barra, G. De Paepe, *J Magn Reson* **2014**, *239*, 91-99; bS. Lange, A. H. Linden, U. Akbey, W. T. Franks, N. M. Loening, B. J. van Rossum, H. Oschkinat, *J. Magn. Reson.* **2012**, *216*, 209-212.

[29] P. C. A. van der Wel, K.-N. Hu, J. Lewandowski, R. G. Griffin, *J. Am. Chem. Soc.* **2006**, *128*, 10840-10846.

[30] aC. I. Ratcliffe, K. Yu, J. A. Ripmeester, M. Badruz Zaman, C. Badarau, S. Singh, *Phys. Chem. Chem. Phys.* **2006**, *8*, 3510-3519; bV. Ladizhansky, G. Hodes, S. Veha, *J. Phys. Chem. B* **1998**, *102*, 8505-8509.

[31] O. N. Antzutkin, S. C. Shekar, M. H. Levitt, *J. Magn. Reson.* **1995**, *115*, 7-19.

Supporting Information

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Frozen Acrylamide Gels as Dynamic Nuclear Polarization Matrices.

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Experimental Procedures

Synthesis of polyacrylamide gel

30% Acrylamide (acry)/N,N'-methylene bisacrylamide(bisacry) solution in water with three different acry/bisacry ratio 37.5:1, 29:1 and 19:1, tetraethyldiamine, and ammonium persulfate were purchased from BioRad and used without further purification. AMUPol was obtained from the Ouari and Tordo group and synthetized according to literature.[1]Deuterium oxide (99.9 atom % D) and acetone (HPLC grade) were purchased from Sigma Aldrich.
Polyacrylamide gels synthesized from acry/bisacry ratio 37.5:1, 29:1 and 19:1, and now referred to as **A**, **B** and **C** were prepared according to the literature.[2] For this study, the synthesis of the gels yields about 500 mg of the desired product. Following this precise order: D_2O (480 µL, 26 mmol) was mixed with 30 % acrylamide: N, N'- methylene bisacrylamide ratio (**A** 37.5:1, **B** 29:1, **C** 19:1) (500 µL), then the initiator 10 % ammonium persulfate (8 µL, 0.8 mmol) and accelerator tetramethyldiamine (1 µL, 6.7 µmol) are added in an Eppendorf. The mixture is immediately vortexed for 30 seconds and left to polymerize for 15 minutes at room temperature. Following the gel formation, it is washed by using the *breathing technique* introduced by Pardo-Yissar et al.[2] This consists of two steps: (i) *collapsing of the gel*: 1 mL of acetone is added to 60 mg of polyacrylamide gel three times to extract/expulse the water along with the impurities APS and TEMED found inside the gel. One can see the gel become white, as shown in Figure S4 (b). That step is followed by (ii) the *swelling* of the gel where 200 µL of D_2O is introduced back inside and left to diffuse for fifteen minutes. D_2O fills the gel which recovers its initial clear state (Figure 4 (a, c)). The breathing cycle is repeated four times. Once the gel is clean, the polarizing agent AMUPol is diffused in the gel. This is achieved by placing the gel in a AMUPol/D_2O solution of the desired concentration (*vide infra*). AMUPol was allowed to diffuse inside the gel for an hour at room temperature at a volume ratio of 1:1 gel to AMUPol in D_2O. In order to get different concentrations of AMUPol in the gel, various concentrations (5, 10, 20, 40, 60 mM) of AMUPol (726 g/mol) in D_2O were used to diffuse AMUPol in the gel. The supernatant is then removed with a micropipette and used to quantify, by ^1H solution NMR, the remaining amount of AMUPol in the supernatant and thus estimate the concentration of AMUPol in the gel.

Dispersion of nanoparticles inside gel

The breathing technique was used to disperse 150 M CdTe-COOH functionalized in D_2O inside 30 mg of gel. Extraction of the D_2O by adding 500 µL of acetone three times was followed by incorporating 20 µL of CdTe-COOH solution to swell the gel and diffused for 15 minutes. This cycle was performed until the total amount of CdTe-COOH solution (145 µL) was incorporated. 30 µL of 5 mM AMUPol/D_2O was diffused in 30 mg of gel for an hour.

DNP Enhanced NMR Spectroscopy

Typically, 30-50 mg of the gel described above was transferred to a 3.2 mm o.d. sapphire rotor and capped with a teflon plug. Data were acquired at either EPFL or CRMN using 263 GHz/400 MHz Avance **I** or **III** HD Bruker DNP solid-state NMR spectrometer, respectively, equipped with a 3.2 mm Bruker triple resonance low temperature magic angle spinning (LTMAS) probe and the experiments were performed at ca. 90-100 K. The sweep coil of the main magnetic field was set for the microwave irradiation occurring at the ^1H positive enhancement maximum of the AMUPol biradical. Enhancement factor, ε, is the ratio of the signal intensity with and without microwaves.

^{13}C DNP Enhanced NMR Spectroscopy.

For ^{13}C NMR ($\nu_L(^{13}C)$ = 100.6 MHz at 9.4 T), the acquisition parameters used for a CPMAS experiment are: 2 s repetition delay, a ^1H π/2 pulse length of 2.5 µs to afford 100 kHz ^1H decoupling using the SPINAL-64 method, a contact time of 3 ms at a spinning frequency of 10 kHz.

^{113}Cd DNP Enhanced NMR Spectroscopy.

For ^{113}Cd NMR ($\nu_L(^{113}Cd)$ = 88.7 MHz at 9.4 T) was referenced to $Cd(CH_3COO)_2$ $2H_2O$ $\delta_{iso}(^{13}C)$ = -14 ppm. The acquisition parameters used for a CP-CPMG experiment are: 3 s repetition delay, a ^1H π/2 pulse length of 2.5 µs to afford 100 kHz ^1H

decoupling using the SPINAL 64 method[3], a contact time of 8 ms. For the CPMG part of the experiment a total of 500 echoes with 160 points per echo were acquired which provides spikelet separation equal to 2500 Hz at a spinning frequency of 10 kHz. 32160 points were acquired in the direct dimension. For the direct excitation CPMG experiment, the same parameters were used with the exception of 750 echoes were acquired, a longer recycle delay was necessary 300 s, and 240160 points were acquired in the direct dimension. The two-dimensional CP sideband separation in MAS (PASS) experiment the same conditions were used for the CP, and 600 steps were used.[4]

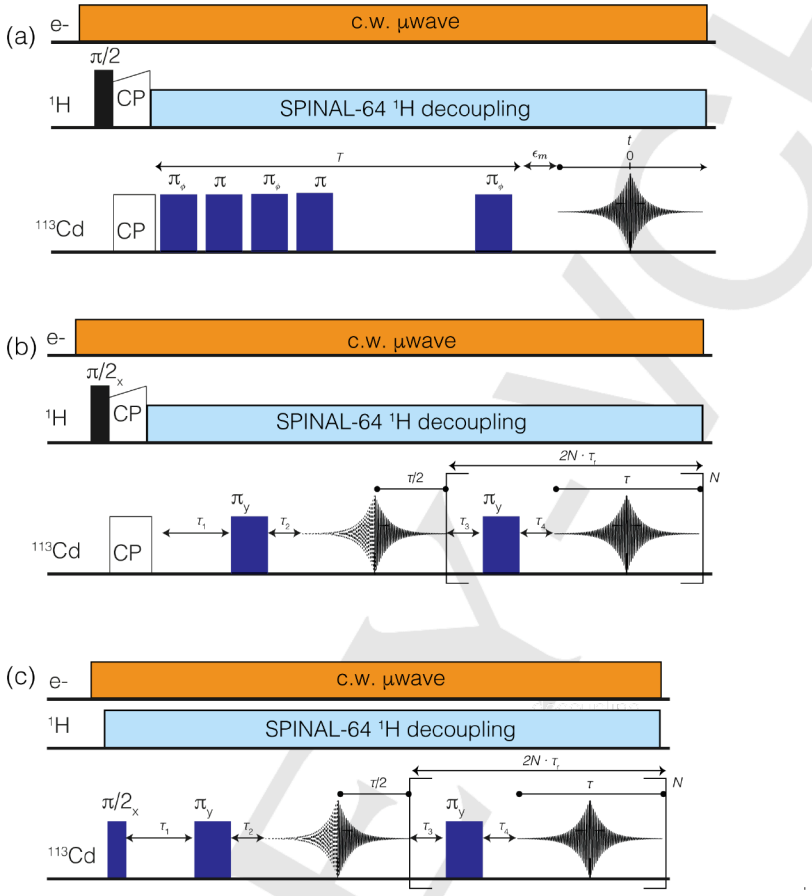

Figure S1. Pulse sequences used to acquire Cd^{113} (I= ½, V_L= 88.73 MHz) spectra, where **(a)** shows the CP-PASS, **(b)** CP-CPMG and **(c)** CPMG.

Quantitative solution NMR experiments.

Quantitative solution NMR experiments have been performed on a 700 MHz Bruker NMR magnet located at CRMN, equipped with an Avance III console and a TXI 5mm $^{1}H/^{13}C/^{15}N/^{2}H$ liquid-state NMR probe.
We used a gel synthetized and washed as described previsously. The acry:bisacry ratio is 19:1 (gel C). After impregnation of 500 µL of gel with 500 µL of a 5 mM AMUPOL solution in D_2O, 250 µL of the supernatant is collected. The latter is first mixed with 300 µL of a solution in D_2O containing 28 mM of ascorbic acid and 1 mM of 4,4-dimethyl-4-silapentane-1-sulfonic acid (DSS). Ascorbic acid is introduced in large excess and reduces the nitroxide radicals of AMUPol. In order to make sure the reaction is complete, the solution is heated at 50°C for 10 minutes. DSS has been weighed precisely on a micro-balance (± 0.5 µg) and used as an internal reference for quantification. We acquired ^{1}H spectra with a total recycling time of 47 s and 128 scans. Remaining water signals were removed using CW saturation of water peak during 4 s at 86 Hz prior to the excitation pulse (^{1}H pulse 34 kHz). Quantification is performed using the DSS peak at 0 ppm corresponding to $Si(C\mathbf{H}_3)_3$ of DSS and the peak at 3.34 ppm assigned to CH_3 of the reduced AMUPol. Resonance assignments of the mixture ascorbic acid, AMUPol (and their respective oxidized and reduced forms) and DSS have been done using a combination of COSY, edited HMQC and DOSY experiments. We found a concentration of AMUPOL in the supernatant of 1.19 mM and thus we can deduce the concentration of AMUPOL in the gel to be 3.81 mM. Reproducibility of this protocol has been tested using a different batch of gel and a second DSS/ascorbic acid solution (same concentration and volume), were we found a concentration of AMUPOL in the gel of 3.80 mM which is in good agreement with our hypothesis of 2.5 mM. Thus, we calculate the partition coefficient $\mathcal{P}$ of AMUPOL between the gel and D_2O:

$$\mathcal{P} = \frac{[\text{AMUPOL}]_{gel}}{[\text{AMUPOL}]_{D_2O}} = 3.2$$

Finally, we can estimate the concentration of AMUPOL in the gel as a function of the concentration of AMUPOL in the solution used to impregnate the gel. Note that for all gel impregnation, we used an equal volume of gel and AMUPOL solution.

Table S1. Summary of final concentrations of opmized DNP Jelly radical concentration determined with quantitative solution NMR.

AMUPOL concentration in the solution used to impregnate the gel (mM)	Actual AMUPOL concentration in gel C (acryl bisacryl 19:1 ratio) (mM)
60	45.7
40	30.5
20	15.2
10	7.6
5	3.8

DNP Data Processing.

DNP enhancements were determined by comparing the integration of the resonance of interest for the spectra acquired with and without microwave using MatLab software v7.10. Proton spin lattice relaxation measurements were measured using saturation recovery experiment. Data are fit using a mono-exponential of the form: $S(\tau) = A\left[1 - m.e^{-\frac{\tau}{T_B}}\right]$; where, A is the equilibrium signal intensity with microwave irradiation, $S(\tau)$ is the integrated intensity at recycle delay time τ, and T_B is the build-up value acquired with μwave off. The CP-CPMG train of echoes were co-added using RMN software and the CP-PASS isotropic dimension was also treated in RMN.

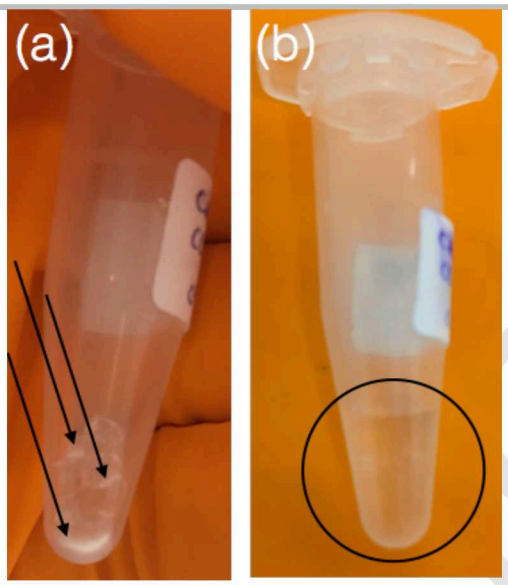

Figure S2. *Breathing technique* used to wash the gel. The freshly synthesized gel is placed in acetone which has the effect to collapse and have a white aspect (shown by black arrows) **(b)**. Then the gel **(a)** is placed in D_2O, and swells back to its original translucent form (b).

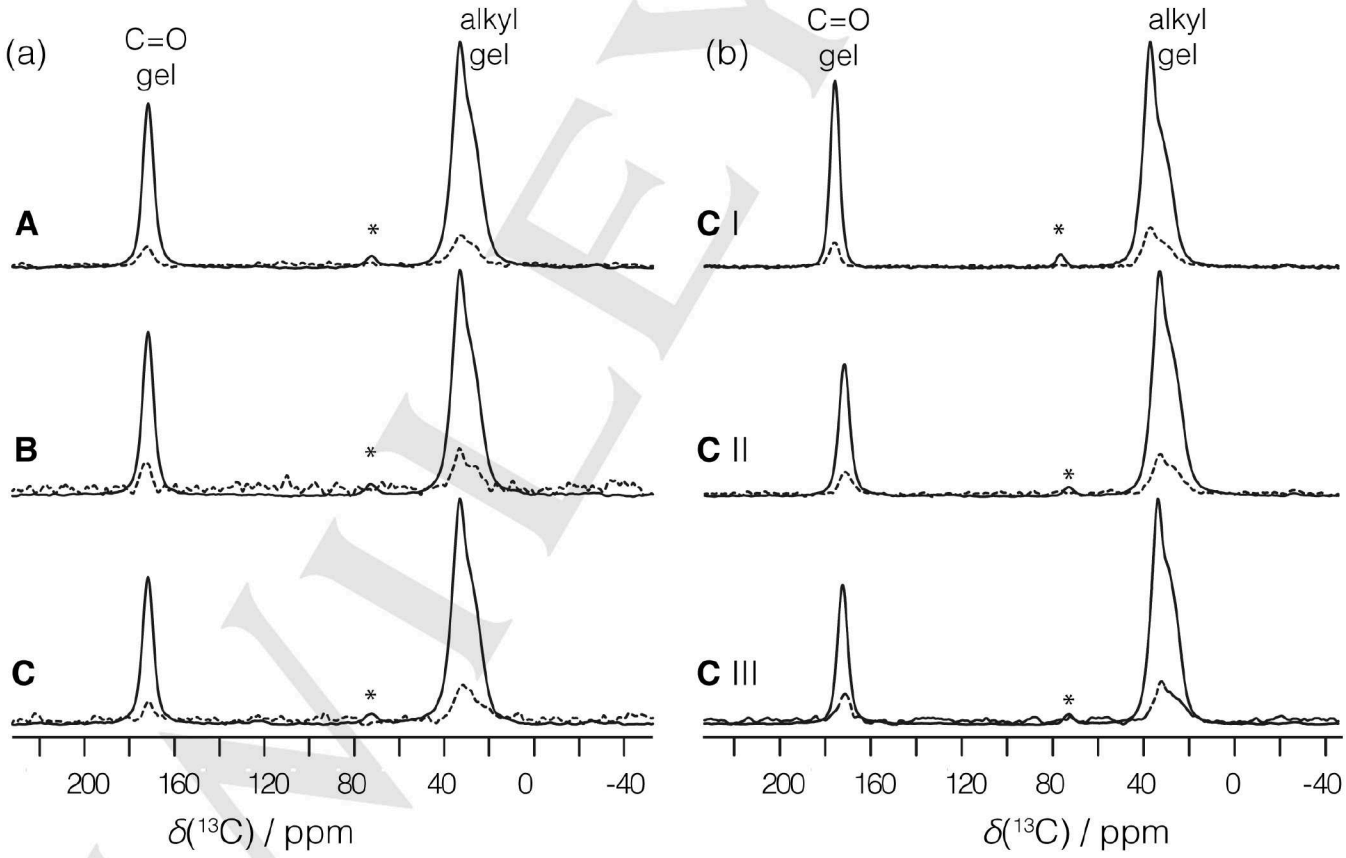

Figure S3. **(a)** ^{13}C CPMAS DNP enhanced spectra of polyacrylamide gels **A**, **B** and **C** after a diffusion step with a 20 mM AMUPol solution in D_2O. The two ^{13}C signals at 30 ppm and 175 ppm were assigned to the alkyl and amide carbons of the polyacrylamide gel. **(b)** Reproducibility tests performed on three different batch (CI, CII and CIII) of polyacrylamide gel **C** after a diffusion step with a 5 mM AMUPol in D_2O. After diffusion, the concentration of AMUPOL is 3.8 mM in the gel. Spectra with microwaves are represented with a solid line whereas spectra recorded without microwaves are plotted with dashed lines. All spectra were acquired with MAS rate set to 10 kHz and a 2 s repetition delay, where * denotes spinning side bands.

SUPPORTING INFORMATION

Furthermore, the ^{1}H relaxation time measured with μwave off (build-up time without μwave irradiation, T_B off) as a function of the AMUPol concentration is plotted in Figure 1 (b). Build-up experiments with μwave off measures the average between short and long spin-lattice relaxation delays of protons in DNP jelly and in water. Protons in close proximity to the radical will have shorter T_B compared to protons situated further away. As the concentration in radical is decreasing, the average relaxation weights less towards the protons in proximity to the radical. Hence, our results follow the expected trend that the T_B decreases with increasing radical concentration as more protons are surrounded by radicals. Our experimental results are in agreement with previously reported studies for different formulations such as TOTAPOL in DMSO-d6/D_2O/H_2O (6/3/1 v/v),[4] TEMPO in glycerol-d8/D_2O (6/4 v/v)[5] or bTbK in tetrachloroethane.

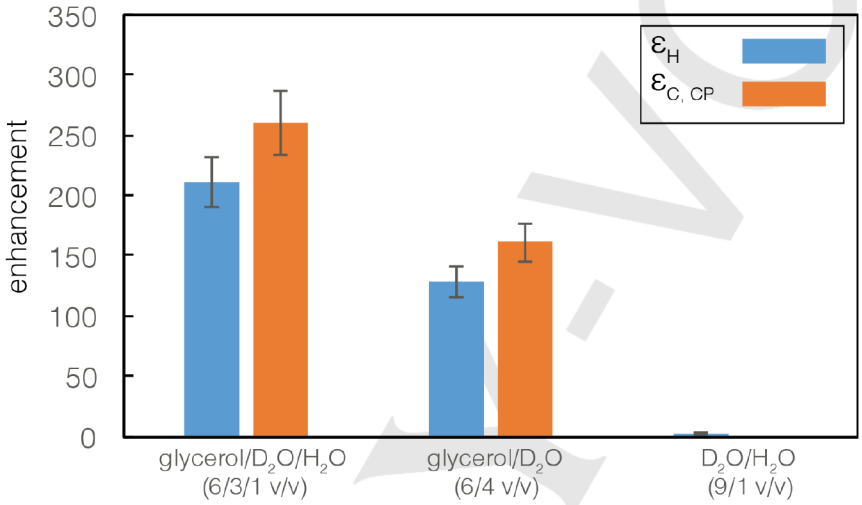

Figure S4. (a) DNP ε_H (blue) and $\varepsilon_{C,\,CP}$ (orange) of 10 mM AMUPol in glycerol-*d*8/D_2O/H_2O (6/3/1 v/v), glycerol-*d*8/D_2O (6/4 v/v) and D_2O/H_2O (9/1 v/v).

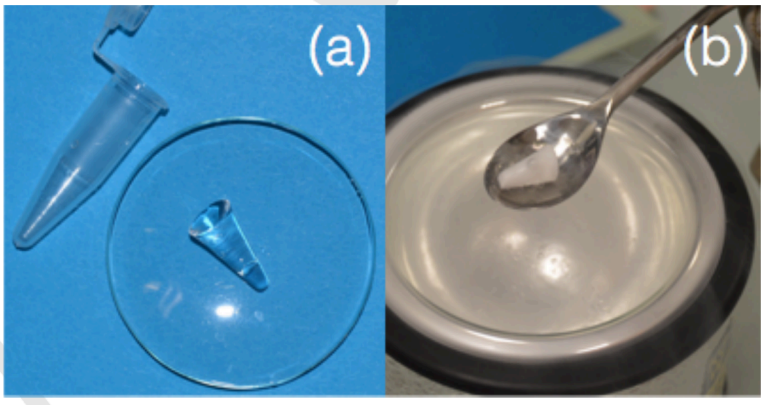

Figure S5. Polyacrylamide gel at (a) room temperature, and (b) after flash frozen in liquid nitrogen. The glassing properties and transition of a polymer gel are not straight forward at 100 K. However, we observe good DNP ε for frozen DNP Jelly. The white opaque color might correspond to micro-domains of water crystalizing. The DNP process is not affected since they are micro-domains, i.e. radical concentrated zones are minimal.

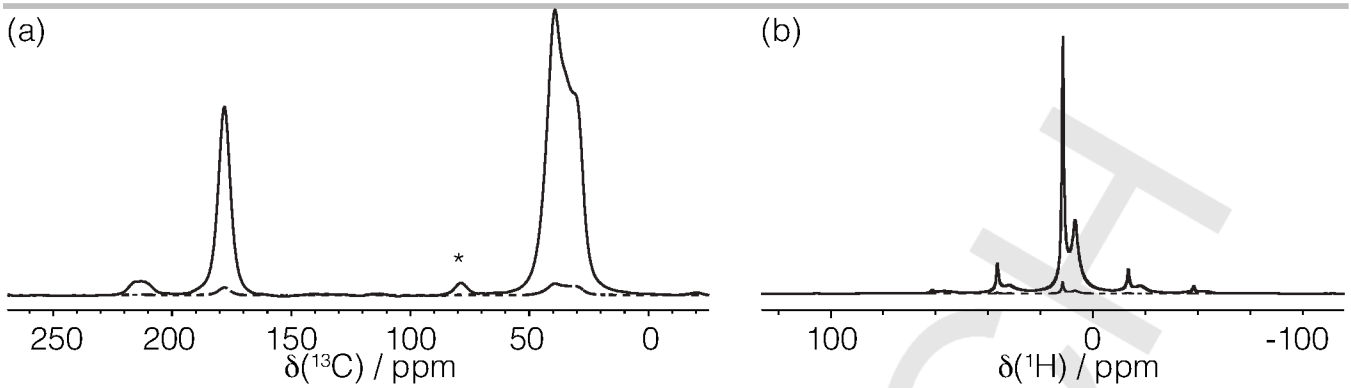

Figure S6. **(a)** ^{13}C CPMAS and **(b)** ^{1}H DNP spectra of polyacrylamide gel **C** with dispersed CdTe-COOH NP acquired with 3.8 mM AMUPol/D_2O. MAS rate was set to 10 kHz and a 2 s repetition delay was used. Spectra with microwaves are represented with a solid line whereas spectra recorded without microwaves are plotted with dashed lines. * denotes spinning side bands. The ^{13}C signal in **(a)** at 213 ppm was assigned to the C=O resonance of acetone (its presences is due to the breathing procedure).

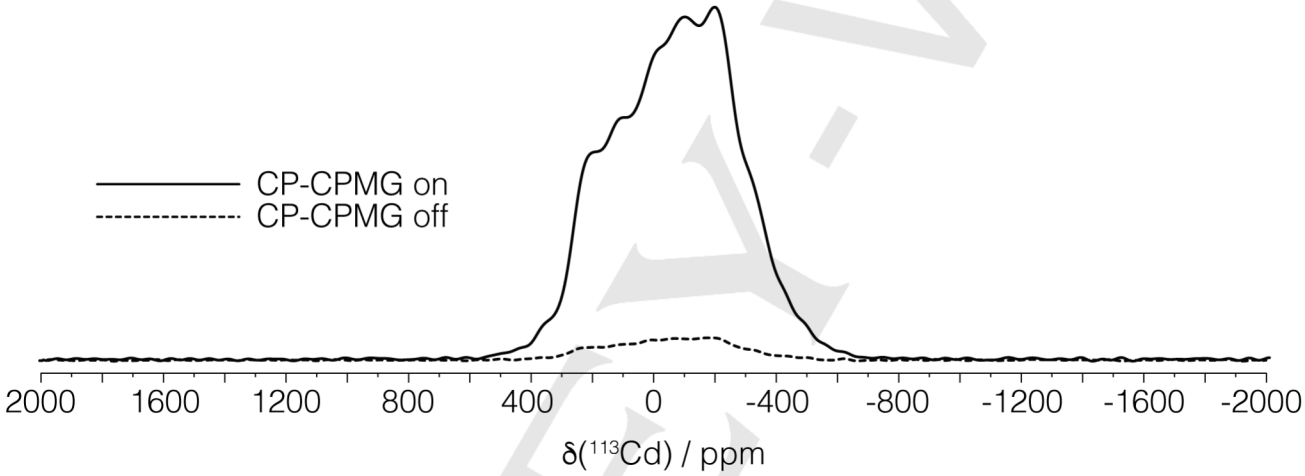

Figure S7. ^{113}Cd DNP CP-CPMG acquired with microwave irradiation on (black) and off spectra of CdTe-COOH NP dispersed in gel **C**. Spinning sidebands can be depicted (MAS of 10 kHz) in the (CP-)CPMG experiments.

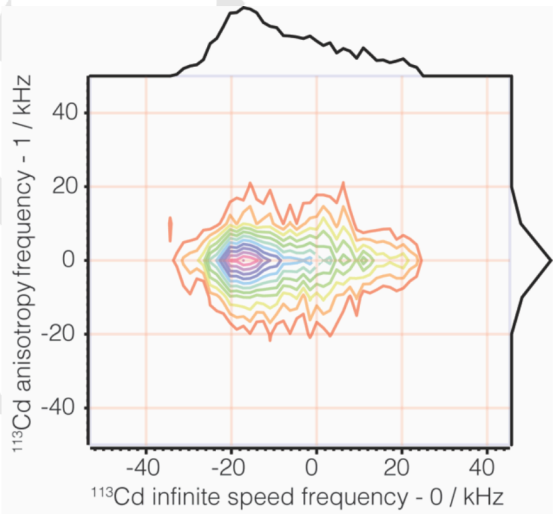

Figure S8. 2D CP-PASS (orange) spectrum of CdTe-COOH NP dispersed in gel **C**.

References

[1] C. Sauvee, M. Rosay, G. Casano, F. Aussenac, R. T. Weber, O. Ouari, P. Tordo, *Angewandte Chemie* **2013**, *52*, 10858-10861.
[2] V. Pardo-Yissar, R. Gabai, S. A. N., B. T., W. I., *Adv. Mater.* **2001**, *13*, 1320-1323.
[3] B. M. Fung, A. K. Khitrin, K. Ermolaev, *J. Magn. Reson.* **2000**, *142*, 97-101.
[4] O. N. Antzutkin, S. C. Shekar, M. H. Levitt, *J. Magn. Reson.* **1995**, *115*, 7-19.